

## Ultrastructure of Spermatozoa in *Plebeia (Plebeia) droryana* Friese (Hymenoptera: Apidae: Meliponina)

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**Abstract.**—In general, the spermatozoa of *Plebeia (Plebeia) droryana* Friese are very similar to those described for other Hymenoptera. However, their arrangement in spermatodesmata bundles in the seminal vesicle has not yet been found in Apidae, this being a characteristic observed, to date, only in Symphyta, the Hymenoptera considered most primitive. The spermatozoa are long and thin, made up of a head connected to the tail at the position of the centriolar adjunct. The head includes an acrosomal vesicle, a perforatorium and a electron dense nucleus. The flagellum consists in a typical axoneme, two mitochondrial derivatives and two accessory bodies. Unlike most other Hymenoptera, the centriolar adjunct is very long and located between the nuclear base and the anterior extremity of the smaller mitochondrial derivative. It has recently been demonstrated that the structure and ultrastructure of hymenopterian spermatozoa are sufficiently varied so as to furnish consistent character matrices that can contribute to phylogenetic studies ("Spermiocladistics"). Since no consensual phylogenetic hypothesis has yet been proposed for Apidae, the data presented here may be a contribution in this direction.

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The Apidae have been extensively studied due to their economic and ecological importance, since they are pollinators, often exclusively, of the majority of flowering plants, including species cultivated by man. The relation between these pollinating agents and the plants they pollinate is so intimate that changes in the biodiversity of either group is certain to affect the other. The Apidae are also recognized as a diverse group with complex social behaviour, which culminate in advanced eusocial societies, a level observed only among Hymenoptera (a few bees and wasps) and in the Isoptera.

Within the Apidae, the tribe Apini, consisting in the subtribes Apina, Bombina, Euglossina and Meliponina (*sensu* Roig-Alsina and Michener 1993), is particularly interesting because its members collectively display all levels of social behaviour. Ranging from solitary bees, as in some Euglossina, to advanced eusocial groups,

such as the Apina and the Meliponina, passing through intermediate social behavior groupings as found in the Bombina and Euglossina.

In spite of the unquestioned importance of the Apidae, so far neither morphological nor molecular studies have been able to establish an uncontested phylogeny for this group (Camargo and Pedro 1992b; Cameron 1991, 1993; Cameron et al. 1992). The establishment of the phylogeny of this group would undoubtedly be important for studies of the evolutionary mechanism, or mechanisms, leading to eusocial behaviour (Crozier and Pamilo 1996).

Structural and ultrastructural characteristics of the spermatozoa, besides their own biological and taxonomic aspects, may be very interesting if this information can be used to form a character matrix for phylogenetic analysis. This information, associated with other character systems, could lead to a better understanding of the

evolutionary relationships within the group ("spermiocladistics", Jamieson 1987) as is being carried out for other animals, including insects (Baccetti 1972; Dallai 1979; Dallai and Afzelius 1990, 1995; Carcupino et al. 1995; Jamieson et al. 1999; Lino-Neto et al. 1999, 2000a, 2000b).

The spermatozoal ultrastructure of only one apid species, *Apis mellifera* Linnaeus, representing the Apini, has so far been studied in detail (Rothschild 1955; Hoage and Kessel 1968; Cruz-Höfling et al. 1970; Lensky et al. 1979; Woyke 1970; Lino-Neto et al. 2000b). Besides this species, in Meliponina only some aspects of spermiogenesis were investigated, including that of *Scaptotrigona postica* Latreille (Cruz-Landim and Beig 1980; Cruz-Landim et al. 1980), *Melipona quadrifasciata anthidioides* Lepeletier (Cruz-Landim et al. 1980; Cruz-Landim and Moraes 1980), *Plebeia* (*Plebeia*) *droryana* Friese, *Friescomelitta* (*Friescomelitta*) *varia* Lepeletier, *Leurotrigona muelleri* Friese (Cruz-Landim et al. 1980). However these publications contain almost no information on the mature sperm cell. Therefore, in this study, we characterize the structure and ultrastructure of *Plebeia* (*Plebeia*) *droryana* sperm so as to furnish data that could be used for future phylogenetic research.

## MATERIAL AND METHODS

Adult males of *Plebeia* (*Plebeia*) *droryana* were obtained from colonies maintained in the Central Apiary of the Federal University of Viçosa, MG, Brazil.

**Light Microscopy.**—Seminal vesicles were dissected and broken open on clean glass microscope slides, where the sperm were spread and fixed in a solution of 4% (wt/vol) paraformaldehyde in 0.1 M phosphate buffer, pH 7.2. After drying at room temperature, the preparations were observed with a photomicroscope (Olympus, BX60), equipped with phase contrast.

To measure the nucleus, some of these preparations were stained for 15 min. with 0.2 µg/ml 4,6-diamino-2-phenylindole

(DAPI) in phosphate buffered saline, washed, and mounted with Vectashield. They were examined with an epifluorescence microscope (Olympus, BX60), equipped with a BP360–370 nm excitation filter.

**Transmission Electron Microscopy.**—Seminal vesicles were dissected and fixed for 3 hours in a solution containing 2.5% glutaraldehyde, 0.2% picric acid, 3% sucrose and 5 mM CaCl<sub>2</sub> in 0.1 M cacodylate buffer, pH 7.2. The materials were post fixed in 1% osmium tetroxide, in the same buffer, for 1–2 hours. Dehydration was carried out in acetone and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate and observed with the Zeiss LEO 906 transmission electron microscope.

## RESULTS

In the seminal vesicle, the spermatozoa of *Plebeia* (*Plebeia*) *droryana* are organized in spermatodesmata bundles, where the anterior region of the heads are embedded in a substance of medium electron density (Figs. 1, 2). The more central spermatozoa are situated slightly ahead of the lateral ones, so that a transverse section of this region shows acrosomes sectioned at different levels (Fig. 2). However, some isolated spermatozoa also appear chaotically dispersed in the seminal vesicles (Figs. 5–7).

The spermatozoan of *P. droryana* is long and thin, measuring approximately 135 µm in length (Fig. 3). The acrosome measuring about 1.2 µm and is made up of the acrosomal vesicle and the perforatorium (Figs. 1, 5–6). The acrosomal vesicle is cone-shaped and covers the perforatorium along its entire length (Fig. 6). In transverse section, the acrosome is circular at the tip but becomes triangular, particularly the perforatorium towards the nucleus (Figs. 2, 8–9). Along the circular portion, an electron transparent layer covers the perforatorium, separating it completely from the acrosomal vesicle. However,

when they are triangular this clear layer is reduced to patches at the vertices (Figs. 8–9). The perforatorium base penetrates about 70 nm into a small asymmetric cavity in the nuclear tip (Fig. 7).

The nucleus measures approximately 7.5  $\mu\text{m}$  in length and is filled homogeneously with dense chromatin. In transverse section, it is slightly oval, measuring approximately 0.18  $\mu\text{m}$  in diameter at the anterior extremity and 0.45  $\mu\text{m}$  at the posterior (Figs. 2–7, 10–13). At the anterior tip there is a cavity in which the perforatorium fits (Fig. 7), while posteriorly the nucleus tapers conically and is covered by thin electron transparent and electron dense material (Figs. 12–13, 15).

The axoneme, measuring 126  $\mu\text{m}$  of length, presents the 9+9+2 pattern of microtubules, with 9 single, external, accessory microtubules, nine doublets and a pair of single ones in the center of the arrangement (Figs. 18–21). In the first 0.28  $\mu\text{m}$ , corresponding to the centriole, the axoneme consists only of the accessory microtubules, the doublets and a dense amorphous substance (Fig. 16). The central microtubules begin posterior to the centriolar portion (Fig. 17). In the final portion, the axoneme is gradually disorganized, with the central microtubules and the nine doublets terminating first, simultaneously, followed by the accessory microtubules (Figs. 21–24).

The centriolar adjunct is very long, about 4.6  $\mu\text{m}$  in length, compact and electron dense. It begins at the nuclear base and extends parallel to the axoneme until it fits onto the smaller mitochondrial derivative (Figs. 11–14). In longitudinal section, it has a rod-like shape while in transverse section it is approximately circular, with a diameter of about 0.2  $\mu\text{m}$  (Figs. 1, 11–12, 14, 16–18).

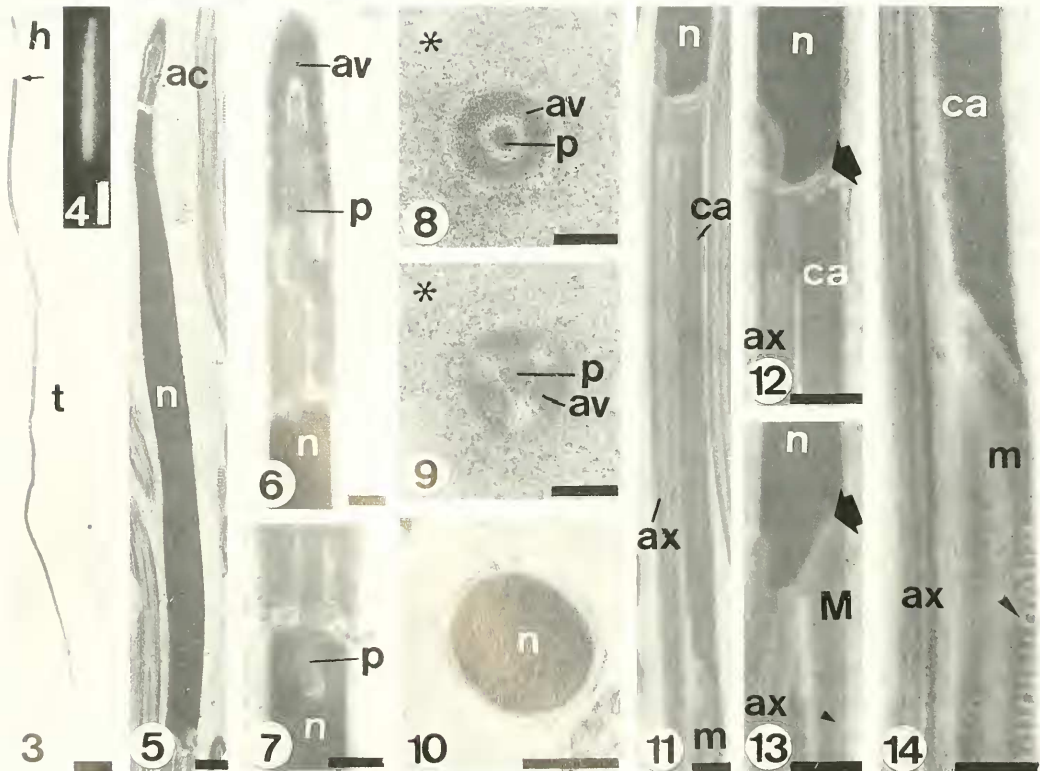
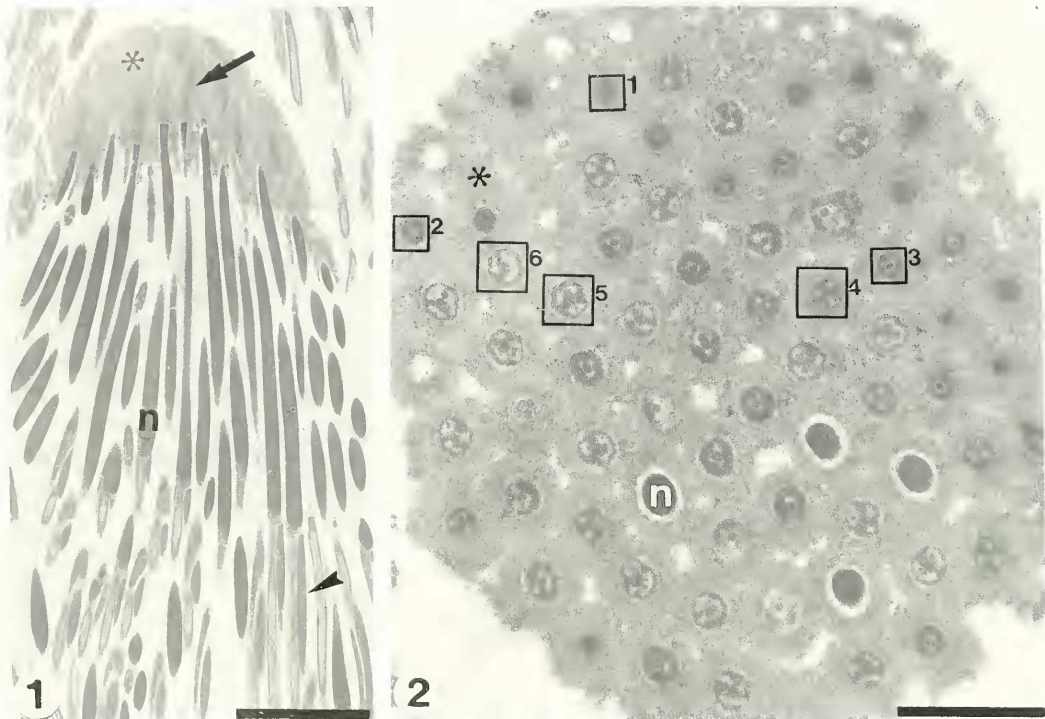
The mitochondrial derivatives are asymmetric in both length and diameter (Figs. 11, 13–14, 19). Anteriorly, the larger mitochondrial derivative begins next to the tapering nucleus (Fig. 13) and the

smaller in contact with the posterior end of the centriolar adjunct. In transverse section, the derivatives are elipsoidal, with the larger one curving slightly over the smaller one (Fig. 19). Both have at least three regions: a dense material that fills in most of the mitochondrial derivatives; a clear approximately central area and the region of the cristae, limited to that part of the periphery opposite the axoneme (a, b and c in Figs. 16–19). The large mitochondrial derivative also has a region of regularly arranged paracrystalline material in the third that is most distal to the axoneme (p in Figs. 16–19). Anteriorly, the derivative extremities do not show any cristae (Figs. 13–14).

The accessory bodies are located laterally, between the axoneme and the mitochondrial derivatives. In transverse sections, they have a triangular shape (Figs. 18–20). In the centriolar adjunct region, there is only one accessory body present between the larger mitochondrial derivative and the axoneme (Fig. 18).

## DISCUSSION

The arrangement of spermatozoa in spermatodesmata observed in *Plebeia droryana*, has not been described for Apocrita. According to Quicke et al. (1992), this spermatozoa arrangement in bundles is characteristic of Symphyta, considered primitive Hymenoptera, in spite of some sheath fragments encountered by these authors in some Aculeata. The central spermatozoa of the sheaths are somewhat ahead of the others, as observed in *P. droryana*, as also occurs in Xyeloidea and Phamphiloidea, which are considered the most basal Symphyta (Newman and Quicke 1999a). However, in the Siricidae, considered the family most closest to Aculeata studied so far, the central spermatozoa are inserted well ahead of the peripheral ones, so that in transverse sections, they are observed in very different levels (Newman and Quicke 1999a). Although most of the spermatozoa are organized in



spermatodesmata in *P. droryana*, as in the symphytans, *Tremex* sp. (Newman and Quicke 1999a) and *Calameuta* sp. (Quicke et al. 1992), some spermatozoa are free. Newman and Quicke (1999a) suggested that the observation of free spermatozoa in the seminal vesicle could be due to fixation or if they indicate a pre-transfer phenomenon. We believe that it is also possible that these spermatozoa have either not yet been grouped into spermatodesmata, or even that not all spermatozoa are destined to become included in bundles.

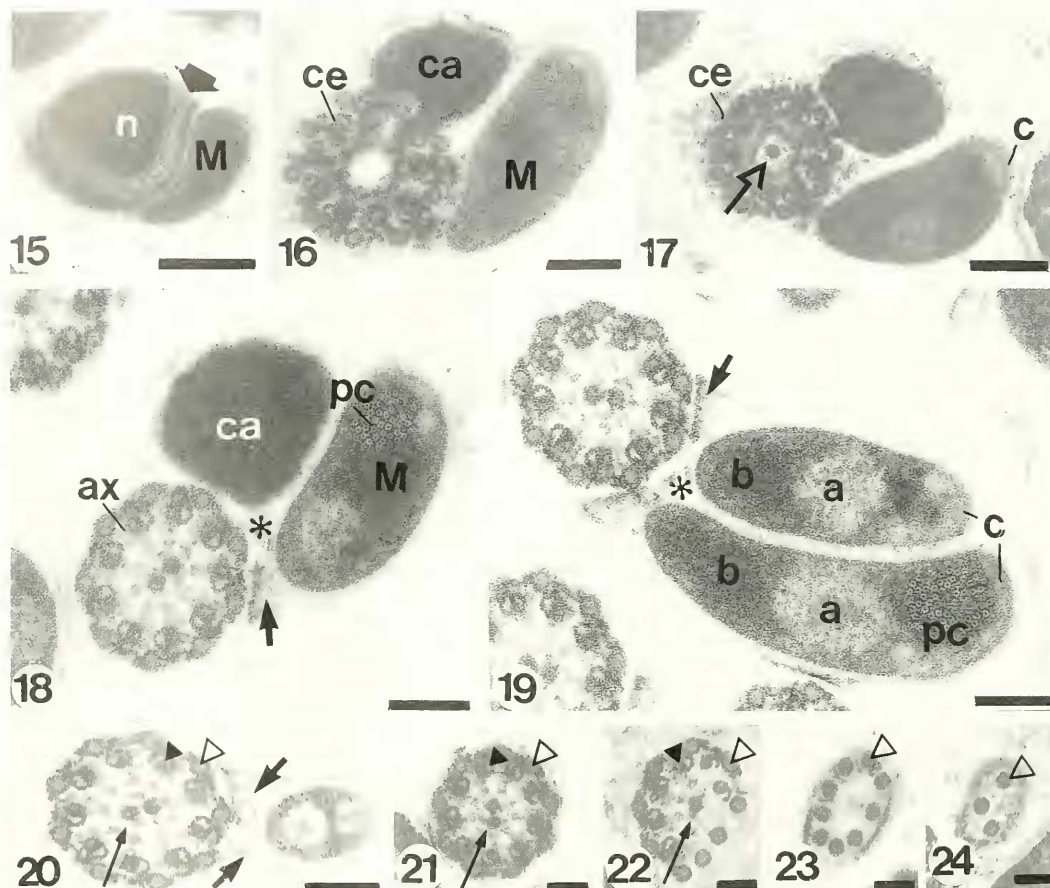
In all the apocritan non-Aculeata (parasitic wasps) considered to date, the spermatozoa appear isolated in the seminal vesicle, and no spermatodesmata fragments have been observed (ex. Quicke et al. 1992; Newman and Quicke 1998, 1999b; Lino-Neto et al. 1999, 2000a; Lino-Neto and Dolder 2001a, b). The fact that spermatozoa organized in spermatodesmata occur in Symphyta and in at least one apocritan Aculeata, which are considered, respectively, the most basal and the most derived hymenopteran groups, while not occurring in the apocritan non-Aculeata, is very intriguing. This suggests either that it could be a reversed character state in Aculeata or that this group derived directly from the Symphyta, as is believed to have occurred with parasitic wasps. This latter hypothesis seems less likely

since morphological and molecular analyses suggest that Aculeata are the sister group of the Ichneumonoidea (Whitfield and Cameron 1998; Ronquist et al. 1999).

The basic structure of the spermatozoa in *P. droryana* is quite similar to that described for other Hymenoptera, as well as for insects in general (Phillips 1970; Bacetti 1972). The acrosome of *P. droryana*, made up of an acrosomal vesicle and the perforatorium appears to be typical for Hymenoptera (Jamieson 1999), having been found in Symphyta (Quicke et al. 1992; Newman and Quicke 1999a), in the Scelionidae, *Trissolcus basalis* (Lino-Neto and Dolder 2000a), in Formicidae (Wheeler et al. 1990) and in *Apis mellifera* (Cruz-Höfling et al. 1970; Hoage and Kessel 1968; Lensky et al. 1979; Peng et al. 1992, 1993). In this last species, unlike the other Hymenoptera studied, the acrosome is almost as long as the nucleus, measuring about 5.6  $\mu\text{m}$ . The fact that the acrosome of *P. droryana* shows a circular cross section at the tip gradually being modified into a triangular form as it reaches towards the nucleus differs from other Hymenoptera where this acrosome are always circular (ex. Symphyta, Newman and Quicke 1999a; Cynipoidea, Newman and Quicke 1999b; Chalcidoidea, Lino-Neto et al. 1999, 2000a; Lino-Neto and Dolder 2001b; Formicidae, Wheeler 1990),

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Figs. 1–14. Ultramicrographs of *Plebeia* spermatozoa in seminal vesicle. 1–2, Longitudinal and transverse sections, respectively, of anterior region of a spermatodesm. 1, Acrosomal region (arrow) and portion of nucleus (n) embedded in less electron dense extracellular material (\*). The arrowhead indicates the centriolar adjunct. 2, Numbers 1–6 indicate acrosomes sectioned in anterior-posterior levels from tip to just above nucleus. 3–4, Phase contrast micrograph of a spermatozoa (3) and head region, DAPI-stained fluorescence of nucleus. The arrow indicates the head (h) and tail (t) limit. 5, Longitudinal section showing acrosome (ac) and nucleus. 6, Longitudinal section of acrosomal vesicle (av) and perforatorium (p). 7, Transition region of acrosome-nucleus showing perforatorium base fitting into cavity of nuclear tip. 8–10, Transverse section of acrosome tip (8), base of acrosome (9) and nucleus free of extra cellular material (10). 11–13, Longitudinal sections of nucleus-flagellum transition region. Arrows indicate connective material at nuclear base (12, 13); 14, Longitudinal section at junction of centriolar adjunct and smaller mitochondrial derivative. Arrowhead indicates mitochondrial cristae. Abbreviations: n = nucleus; ac = acrosome; av = acrosomal vesicle; p = perforatorium; ca = centriolar adjunct; ax = axoneme; M = larger mitochondrial derivative; m = smaller mitochondrial derivative. Scale bar: 1, 4, 8–9 = 3  $\mu\text{m}$ ; 2 = 2  $\mu\text{m}$ ; 3 = 8  $\mu\text{m}$ ; 6 = 0.1  $\mu\text{m}$ ; 7 = 0.2  $\mu\text{m}$ ; 10–11, 14 = 0.3  $\mu\text{m}$  and 5, 12–13 = 0.5  $\mu\text{m}$ .



Figs. 15–24. Sequential transverse sections of flagella. 15, Nucleus-flagellum transition region. Arrow indicates material connecting nucleus to larger mitochondrial derivative. 16–17, Centriolar region of axoneme. Open arrow indicates first of central microtubules. 18–19, Sections of flagellum, at centriolar adjunct region and at both mitochondrial derivatives, respectively. The arrows indicate accessory bodies and (\*) indicates central material between flagellar structures. 20–24, Final flagellar region. The nine doublets (arrowheads) and two central microtubules (small arrow) terminate first, followed by accessory ones (white arrowheads). Large arrows indicate accessory bodies. Abbreviations: a = less electron dense amorphous region; b = more electron dense amorphous region; c = cristae region; pc = paracristalline region in the larger mitochondrial derivative; ca = centriolar adjunct; ce = centriole; n = nucleus; ax = axoneme. Scale bar: 15–20 = 0,1 $\mu$ m; 21–22 = 0,06 $\mu$ m and 23–24 = 0,05 $\mu$ m.

or maintains an oval cross section as in *Apis mellifera* (q.v.) and Vespidae (personal observation). The acrosome of *A. mellifera* also differs from that of *P. droryana* due to the presence of a long anterior projection of the acrosomal vesicle (Cruz-Höfling et al. 1970; Hoage and Kessel 1968). The penetration of the perforatorium in the nuclear tip as occurs in *P. droryana* has been described for the majority of the hymenopterans (ex. Quicke et al. 1992; Newman

and Quicke 1999a; Wheeler et al. 1990). However, in Eurytomidae, *Beprhatelloides pomorum* Fabricius (Lino-Neto et al. 1999), and in the Pteromalidae, *Nasonia vitripennis* Walker (Hogge and King 1975), the perforatorium base is concave and has the same diameter as the nucleus in this region, fitting directly onto the anterior nuclear surface. In the majority of parasitic wasps, there is a third extracellular layer (the extracellular sheath), covering all of

the acrosome and extending along a variable length of the nucleus (Quicke et al. 1992; Newman and Quicke 1999b; Quicke et al. 1992; Newman and Quicke 1998; Quicke et al. 1992; Lino-Neto et al. 1999, 2000a; Lino-Neto and Dolder 2001b). Also, in some of these, the extra-cellular sheath gives rise to innumerable filaments, probably representing a well developed glycocalyx (Lino-Neto et al. 1999, 2000a; Lino-Neto and Dolder 2001b).

In *P. droryana*, the nucleus is long, dense and usually appears homogeneously compacted. These characteristics are highly conserved in Hymenoptera, and the variations observed have been in length and in the fact that this structure may be linear (ex. Quicke et al. 1992; Jamieson et al. 1999; Wheeler et al. 1990; Lino-Neto et al. 2000b), or twisted in a spiral, as in Chalcidoidea (Lee and Wilkes 1965; Hogge and King 1975; Quicke et al. 1992; Lino-Neto et al. 1999, 2000a, 2000b; Lino-Neto and Dolder 2001b), Scelionidae (Lino-Neto and Dolder 2001a) and Diapriidae (Quicke, personal communication). The nucleus of *P. droryana* ends in a short cone, next to the anterior tip of the large mitochondrial derivative, and terminating in contact with the centriolar adjunct and axoneme. In *Apis mellifera*, the final nuclear projection is considerably longer and inserted in the axoneme, so that in cross section the nucleus is found surrounding the tips of the centriolar microtubules (Peng et al. 1993; Lino-Neto et al. 2000b). In the majority of the Hymenoptera, the nucleus is not tapered posteriorly but instead is abruptly truncated (Quicke et al. 1992; Newman and Quicke 1999a; Newman and Quicke 1999b; Quicke et al. 1992; Wheeler et al. 1990; Lino-Neto et al. 1999, 2000a; Lino-Neto and Dolder 2001b).

The centriolar adjunct of *P. droryana* is a well developed structure located parallel to the axoneme and between the nucleus and the smaller mitochondrial derivative. This arrangement has also been found in some Symphyta (Newman and

Quicke 1999a), Cynipoidea (Newman and Quicke 1999b), Ichneumonoidea (Quicke et al. 1998) and in *A. mellifera* (Lino-Neto et al. 2000b). However, in the Ichneumonoidea this structure is comparatively short (Quicke et al. 1998) while in *A. mellifera*, it is extremely long, tapered anteriorly, widening into a thick rod posteriorly (Lino-Neto et al. 2000b). In the symphytan *Tremex* sp. (Newman and Quicke 1999a) and in the Formicidae (Wheeler et al. 1990), the centriolar adjunct lies between the nucleus and both mitochondrial derivatives. On the other hand, in Chalcidoidea (Lino-Neto et al. 1999, 2000a; Lino-Neto and Dolder 2001b) the centriolar adjunct is located laterally to the final portion of the nucleus, surrounding the nuclear-flagellum transition and extending parallel to the axoneme for a short distance, above the insertion of both mitochondrial derivatives. Contrary to the majority of these insects, no centriolar adjunct was encountered in Scelionidae (Lino-Neto and Dolder 2001a). The great variation in shape and location of the centriolar adjunct, differing from that known for most insects (Jamieson 1982) is probably the reason for the earlier misinterpretations of this element in various Hymenoptera (Cruz-Höfling et al. 1970; Quicke et al. 1992).

The mitochondrial derivatives of *P. droryana* are asymmetric not only in length but also in diameter. As a rule, the derivatives are straight (ex. Quicke et al. 1992; Jamieson et al. 1999; Wheeler et al. 1990; Lino-Neto et al. 2000b), but in Chalcidoidea (Lee and Wilkes 1965; Hogge and King 1975; Quicke et al. 1992; Quicke 1997; Lino-Neto et al. 1999, 2000a, 2000b; Lino-Neto and Dolder 2001b), Scelionidae (Lino-Neto and Dolder 2001a) and Diapriidae (Quicke, personal communication) they spiral around the axoneme. The larger mitochondrial derivative beginning next to the final projection of the nucleus was also observed in *A. mellifera* (Lino-Neto et al. 2000b) and in Cynipoidea

(Newman and Quicke 1999b). This is not the case of the majority of the Hymenoptera, where the larger mitochondrial derivative abuts the nuclear base, not overlapping it (ex. Quicke et al. 1992; Wheeler et al. 1990; Jamieson et al. 1999; Newman and Quicke 1999a). In Megalyroidea (Newman and Quicke 2000), Diapriidae (Quicke, personal communication) and Scelionidae (Lino-Neto and Dolder 2001a) the large mitochondrial derivative projects parallel to the nucleus for a considerable distance, and in this latter family, only one large mitochondrion is observed (Lino-Neto and Dolder 2001a). In transverse sections of the *P. droryana* flagellum, four distinct regions make up the larger derivative while only three are found in the smaller one. The same organization was observed in *A. mellifera* (Lino-Neto et al. 2000b) although Cruz-Höfling et al. (1970), Lensky et al. (1979) and Peng et al. (1992, 1993) have described the presence of paracrystalline material also in the smaller derivative. In the Formicidae the mitochondrial derivatives consist in three regions (Wheeler et al. 1990). However, the regions described in Formicidae are not analogous to those in the smaller derivative of *P. droryana*. In Formicidae, there is a clear area, a well developed region of cristae and the paracrystalline material situated in the mitochondrion's first third, proximal to the axoneme (Wheeler et al. 1990). Asymmetrical diameters of mitochondrial derivatives are frequently found, occurring in the Symphyta (Quicke et al. 1992; Newman and Quicke 1999a), Cynipoidea (Quicke et al. 1992; Newman and Quicke 1999b), Megalyroidea (Newman and Quicke 2000) and Proctotrupoidea (Quicke et al. 1992). However, bees are even more strongly asymmetrical (Cruz-Höfling et al. 1970; Hoage and Kessel 1968; Lensky et al. 1979; Peng et al. 1992, 1993; Lino-Neto et al. 2000b). On the other hand, some Hymenoptera have symmetrical mitochondrial derivatives as in Formicidae (Wheeler et al. 1990) and

Chalcidoidea (Lino-Neto et al. 1999, 2000a).

*Plebeia droryana*, as is common to most insects (Jamieson et al. 1999), has an axoneme with the microtubules arranged parallel to each other. This is not the case of Chalcidoidea (Lee and Wilkes 1965; Hogge and King 1975; Quicke et al. 1992; Quicke 1997; Lino-Neto et al. 1999, 2000a, 2000b; Lino-Neto and Dolder 2001b), Scelionidae (Lino-Neto and Dolder 2001a) and Diapriidae (Quicke, personal communication) where they follow a spiraling course. Also in *P. droryana*, the accessory microtubules are the last ones to terminate at the end of the axoneme. This characteristic is also observed in *A. mellifera* (Peng et al. 1993; Lino-Neto et al. 2000b) and in Formicidae (Wheeler et al. 1990), while in Chalcidoidea (Lino-Neto et al. 1999; Lino-Neto and Dolder 2000a, b) and Ichneumonidae (Braconidae) (Newman and Quicke 1998) the accessory tubules terminate first. Unfortunately, this characteristic has not been taken in consideration by most studies of hymenopteran spermatozoa. We believe this could be a useful parameter to help separate the Aculeata, or parasitic wasps, from other Hymenoptera.

The triangularly shaped accessory bodies, as found in transverse sections of *P. droryana*, are encountered in most Hymenoptera (Quicke et al. 1992; Jamieson et al. 1999; Wheeler et al. 1990; Lino-Neto et al. 2000b). They may be considerably reduced in Chalcidoidea (Lino-Neto et al. 1999, 2000a; Lino-Neto and Dolder 2001b) and in the Scelionidae (Lino-Neto and Dolder 2001a) so that, in some cases, they are difficult to identify. The function of this structure has not been clearly established but they appear to be involved in the attachment of the mitochondrial derivatives on to the axoneme, since they do not occur between the centriolar adjunct and the axoneme.

In *P. droryana* a small central structure was identified between both the mitochondrial derivatives and the axoneme

(see asterisk in Figs. 18 and 19). This structure was initially described in Formicidae (Wheeler et al. 1990), but it is possible that it is present in the majority of Hymenoptera (Lino-Neto et al. 2000b).

Based on the characteristics compared above, the spermatozoa of this bee are, for the most part, similar to the majority of the Hymenoptera (Jamieson et al. 1999). Some distinct differences stand out. For example: (1) the arrangement of spermatozoa in spermatodesmata in the seminal vesicle, (2) the presence of a very long centriolar adjunct between the nucleus and the smaller mitochondrial derivative and (3) the presence of abundant paracrystalline material, exclusively in the large mitochondrial derivative.

The identification of these characteristics and other more subtle ones suggest that the sperm cell can furnish a character matrix for Hymenoptera that will be useful for future phylogenetic studies.

## ACKNOWLEDGMENTS

The authors would like to thank Professor Dr. Lucio Antonio de Oliveira Campos (DBG/UFV) for supplying the insects. This research was supported by the Brazilian Agencies CNPq and FAPESP.

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